Comparative evaluation of growth pattern of selected *Leptospira* serovars cultured on two semi-solid media.

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Introduction and Objectives: Leptospirosis is a neglected tropical zoonotic disease caused by pathogenic *Leptospira*. Effective isolation and maintenance of the cultures of *Leptospira* are crucial for diagnosis, surveillance, and research purposes. Several specialised culture media for long-term maintenance have been developed due to the fastidious nature of the organism. This study compared the growth pattern of few selected *Leptospira* serovars on Ellinghausen-McCullough-Johnson-Harris (EMJH) semi-solid medium and a Leptospira Vanaporn Wuthiekanun (LVW) medium.

Methods: A total of 11 *Leptospira* isolates were tested, including 5 WHO-recommended strains (*Leptospira interrogans* serovars Bratislava, Canicola, Mankarso, and Weerasinghe; *Leptospira borgpetersenii* serovar Ceylonica), five locally isolated *Leptospira* between 2016–2019 (*Leptospira interrogans* serogroups Pyrogenes, Autumnalis, and Ceylonica; *Leptospira weilii* serogroup Celledoni; *Leptospira kirschneri* serogroup Grippotyphosa) and *Leptospira biflexa* serovar Patoc. Standard protocols were followed in the preparation of EMJH and LVW media. Sterile rabbit serum was an additional ingredient added to LVW medium compared to EMJH medium. Both media were inoculated in six replicates per isolate, and all cultures were incubated aerobically at 30 °C. Tubes were monitored daily for one month to assess growth, as indicated by the formation of Dinger's disk at the sub-surface level of the medium. At the end of one month, all cultures were examined under a dark-field microscope for motility and to detect contamination. Results were analysed using SPSS version 23.

Results: Dinger's Disc formed on Day 1 in all LVW cultures, whereas EMJH required a median of 4 days (IQR 4–4) (Mann–Whitney U, $p<0.001$). Disc retention persisted for 30 days in LVW, compared to a median of 7 days (IQR 6–26) in EMJH ($p<0.001$). None of the discs disappeared in LVW (0/66), while disappearance was observed in the majority of EMJH cultures (60/66) (Fisher's Exact, $p<0.001$). Among EMJH cultures where disappearance occurred, the median time to disappearance was 13 days (IQR 10–15). All isolates in both media demonstrated motility at day 30 without any contamination.

Conclusion: LVW agar demonstrated more consistency and sustained growth under the tested conditions compared to EMJH semi-solid medium, highlighting its potential as a reliable medium for long-term cultivation.

Keywords*: Culture media, EMJH, Leptospira, LVW agar*

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